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MicroCorrespondence

Unified nomenclature for broadly conserved *hrp* genes of phytopathogenic bacteria

Sir,

Genes of plant-pathogenic bacteria controlling hypersensitive response (HR) elicitation and pathogenesis were designated '*hrp*' by Lindgren *et al.* in 1986 (*J Bacteriol* 168: 512–522). *hrp* genes have been characterized in several species of the four major genera of Gram-negative plant pathogens, *Erwinia*, *Pseudomonas*, *Ralstonia* (a new proposed genus including *Pseudomonas solanacearum*) and *Xanthomonas*. To date, *hrp* genes have been found mainly in large clusters, and they have been shown to be conserved physically and, in many cases, functionally among different bacteria. Hybridization studies and genetic analyses have revealed the presence of functional *hrp* genes even in species that are not typically observed to elicit an HR, such as *Erwinia chrysanthemi* and *Erwinia stewartii*, suggesting that *hrp* genes may be common to all Gram-negative plant pathogens, possibly excluding *Agrobacterium* spp. Current knowledge of *hrp* genes has been reviewed by Bonas (1994, *Curr Top Microbiol Immunol* 192: 79–98) and by Van Gijsegem *et al.* (1995, In *Pathogenesis and Host–Parasite Specificity in Plant Diseases: Histopathological, Biochemical, Genetic and Molecular Basis*. Volume 1. (Kohmoto *et al.*, eds); Oxford: Pergamon Press, pp. 273–292).

The nucleotide sequences of four *hrp* gene clusters, those of *Ralstonia solanacearum* (previously *P. solanacearum*) (Genin *et al.*, 1992, *Mol Microbiol* 6: 3065–3076; Gough *et al.*, 1992, *Mol Plant–Microbe Interact* 5: 384–389; Gougi *et al.*, 1993, *Mol Gen Genet* 239: 378–392; Van Gijsegem *et al.*, 1995, *Mol Microbiol* 15: 1095–1114), *Erwinia amylovora* (Bogdanove *et al.*, 1996, *J Bacteriol* 178: 1720–1730; Wei and Beer, 1993, *J Bacteriol* 175: 7958–7967; Wei and Beer, 1995, *J Bacteriol* 177: 6201–6210; Wei *et al.*, 1992, *Science* 257: 85–88; S. V. Beer, unpublished), *Pseudomonas syringae* pv. *syringae* (Huang *et al.*, 1992, *J Bacteriol* 174: 6878–6885; Huang *et al.*, 1993, *Mol Plant–Microbe Interact* 6: 515–520; Huang *et al.*, 1995, *Mol Plant–Microbe Interact* 8: 733–746; Lidell and Hutcheson, 1994, *Mol Plant–Microbe Interact* 7: 488–497; Preston *et al.*, 1995, *Mol Plant–Microbe Interact* 8: 717–732; Xiao *et al.*, 1994, *J Bacteriol* 176: 1025–1036), and *Xanthomonas campestris* pv. *vesicatoria* (Fenselau *et al.*, 1992, *Mol Plant–Microbe Interact* 5: 390–396; Fenselau and Bonas, 1995, *Mol Plant–Microbe Interact* 8: 845–854; U. Bonas, unpublished), have been largely determined. These clusters each contain more than twenty genes, many of which encode components of a novel protein-secretion pathway designated 'type III'. It has been shown directly that various extracellular proteins involved in pathogenesis and defence elicitation by plant-pathogenic bacteria utilize this pathway (Arlat *et al.*, 1994, *EMBO J* 13: 543–553; He *et al.*, 1993, *Cell* 73: 1255–1266; Wei and Beer, 1993, Ibid.), and the pathway is known to function in the export of virulence factors from the animal pathogens *Salmonella typhimurium*, *Shigella flexneri*, and *Yersinia enterocolitica*, *Yersinia pestis*, and *Yersinia pseudotuberculosis* (for reviews, see Salmond and Reeves, 1993, *Trends Biochem Sci* 18: 7–12; and Van Gijsegem *et al.*, 1993, *Trends Microbiol* 1: 175–180). Nine type III secretion genes are conserved among all four of the plant pathogens listed above and among the animal pathogens. Based on sequence analysis and some experimental evidence, they are believed to encode one outer-membrane protein, one outer-membrane-associated lipoprotein, five inner-membrane proteins, and two cytoplasmic proteins, one of which is a putative ATPase. All of the predicted gene products, except the outer-membrane protein, show significant similarity to components of the flagellar biogenesis complex (for reviews see Blair, 1995, *Annu Rev Microbiol* 49: 489–522; and Bischoff and Ordal, 1992, *Mol Microbiol* 6: 23–28). We herein refer to the *hrp*-encoded type III pathway as the 'Hrp pathway'.

Because *hrp* genes have been characterized independently in diverse plant-pathogenic bacteria, *hrp* gene nomenclature differs in different species, and it is not always consistent even within the same organism. Different designations are used for homologous genes, and, even worse, the same designation is used for different genes in different organisms. For example, *hrpI* of *E. amylovora* is homologous with *hrpC2* of *X. campestris* pv. *vesicatoria* and *hrpO* of *R. solanacearum*, and the homologue in *P. syringae* pv. *syringae* appears in the literature both as *hrpI* and as *hrpJ2*. Also, '*hrpN*' in *R. solanacearum* designates a secretion-pathway gene, whereas in *E. amylovora*, '*hrpN*' designates the gene encoding the elicitor harpin. Furthermore, in many bacteria the number of known *hrp* genes approaches 26. In anticipation of exhausting the alphabet, some authors chose to designate *hrp* genes with a letter and a number, creating the potential for confusion of distinct genes with alleles of the same gene. For *hrp* gene researchers, the current nomenclature is at best inconvenient; for other scientists, it is bewildering.

Another problem exists: accumulation of knowledge about the structure of *hrp* loci has outpaced the accumu-

lation of information regarding the specific functions of individual genes. Typically, *hrp* loci have been identified by polar, transposon mutagenesis. Conceivably, a particular gene within an operon required for the Hrp phenotype may not be a strict Hrp determinant, but may play a more subtle role. Moreover, even phenotypes of mutations in well-characterized *hrp* genes are not the same in all pathogens. For example, although the *hrpN* gene of *E. amylovora* is required for pathogenesis of pear fruit, the homologous gene in *E. stewartii* (D. L. Coplin, unpublished) is dispensible for pathogenicity of corn. In the macerogenic bacterium *E. chrysanthemi*, even polar mutations that disrupt *hrp* secretion altogether only reduce the apparent frequency of successful infection initiation (Bauer *et al.*, 1994, *Mol Plant-Microbe Interact* 7: 573–581). Thus, the designation '*hrp*' in its strict sense, i.e., meaning required for the HR and pathogenicity, is not uniformly applicable.

At the 7th International Congress on Molecular Plant–Microbe Interactions held in Edinburgh, Scotland in 1994, a committee of *hrp* researchers and others was formed to address these problems. We, the committee members, agreed upon a system to standardize names for the subset of *hrp* genes that are broadly conserved, and agreed to broaden the definition of the '*hrp*' designation, as follows.

For the subset of *hrp* genes that are broadly conserved, the new, unique, lower-case symbol '*hrc*' will be used. The '*hr*' of *hrp* has been retained in order to evoke that name, and the '*c*' has been added to denote 'conserved.' The upper-case designations will correspond to those of the type III secretion genes of *Yersinia* spp. (for a review, see Forsberg *et al.*, 1994, *Trends in Microbiol* 2: 14–19), *yscC*, *yscJ*, *yscN*, *yscQ–U*, and *lcrD*, except that the *lcrD* homologues will be designated '*hrcV*' to avoid confusion of these as homologues of *yscD*, which is another, less well-conserved type III gene of *Yersinia* spp. We request that *Yersinia* researchers omit the letter '*V*' in naming any new *ysc* genes that might be discovered. The *ysc* nomenclature was chosen as a standard for revising *hrp* gene names for its convenient uniformity, and because, of all the genes that comprize the several known type III systems, the *Yersinia* genes show the highest degree of sequence similarity to the type III (*hrp*) genes of plant pathogens. The new names for the nine genes are given in Table 1, along with the current names in *R. solanacearum*, *E. amylovora*, *P. syringae* pv. *syringae*, and *X. campestris* pv. *vesicatoria*, and the names of homologues involved in flagellar biogenesis.

In designating genes as '*hrc*', 'broadly conserved' genes were defined as being present among the *hrp* genes of at least one representative species of each of the four plant-pathogenic genera discussed here and among the type III genes of each of the animal-pathogenic species *S. typhimurium*, *S. flexneri*, and the three yersiniae. Gene families were defined based on pairwise sequence alignments. Any two genes were considered homologous if a segment alignment (Devereux *et al.*, 1984, *Nucl Acids Res* 12: 387–395) of the predicted amino acid sequences using default parameters yielded a quality score at least five times the standard deviation above the mean quality score of 100 alignments, for each of which one of the sequences had been randomized prior to alignment (Doolittle, 1986, *Of URFs and ORFs: a Primer on How to Analyse Derived Amino Acid Sequences*. Mill Valley, California: University Science Books).

Genes that did not meet the criterion for the '*hrc*' designation will remain '*hrp*'. We have chosen to use this criterion until more data regarding structure and precise function of the products of the *hrp* and other type III genes becomes available. Some of the genes that did not meet the criterion in fact may be common to *Ralstonia*, *Erwinia*, *Pseudomonas*, *and Xanthomonas*, and have homologues in the animal pathogens, yet may be sufficiently diverged to obscure obvious homology by direct sequence comparison. As structural and functional data accrue, such relationships may become clear, and the list of *hrc* genes

Table 1. Current names and new, unified names for the broadly conserved *hrp* genes of *R. solanacearum*, *E. amylovora*, *P. syringae* pv. *syringae*, and *X. campestris* pv. *vesicatoria*. Homologues that function in flagellar biogenesis are given in the bottom row.

Unified	*hrcC*	*hrcJ*	*hrcN*	*hrcO*	*hrcR*	*hrcS*	*hrcT*	*hrcU*	*hrcV*
R. solanacearum[a]	*hrpA*	*hrpI*	*hrpE*	*hrpQ*	*hrpT*	*hrpU*	*hrpC*	*hrpN*	*hrpO*
E. amylovora[b]	*hrcC*	*hrcJ*	*hrcN*	*hrcQ*	*hrcR*	*hrcS*	*hrcT*	*hrcU*	*(hrpI) hrcV*
P. syringae[c]	*hrpH*	*hrpC*	*hrpJ4*	*hrpU2/U*	*hrpW*	*hrpO*	*hrpX*	*hrpY*	*(hrpJ2) hrpI*
X. campestris[d]	*hrpA1*	*hrpB3*	*hrpB6*	*hrpD1*	*hrpD2*	*hrpD3*	*hrpB2*	*hrpC1*	*hrpC2*
(Flagellar)[e]		*flF*	*flI*	*flY,N*	*flP*	*flQ*	*flR*	*flhB*	*flhA*

a. Gough *et al.*, 1992, Ibid.; Gough *et al.*, 1993, Ibid.; Van Gijsegem *et al.*, 1995, Ibid.
b. Bogdanove *et al.*, 1996, Ibid.; Wei and Beer, 1993, Ibid.; S. V. Beer, unpublished.
c. Huang *et al.*, 1992, Ibid.; Huang *et al.*, *Mol Plant-Microbe Interact* 6: 515–520, 1993; Huang *et al.*, 1995, Ibid.; Lidell and Hutcheson, *Mol Plant-Microbe Interact* 7: 488–497, 1994; Preston *et al.*, 1995, Ibid. The predicted product of *hrpU2* aligns with the N-terminal two-thirds of a multiple alignment of the other plant- and animal-pathogen homologues; that of *hrpU* aligns with the remaining N-terminal one-third. Respectively, these genes will be designated '$hrcQ_A$' and '$hrcQ_B$.'
d. Fenselau *et al.*, 1992, Ibid.; Fenselau and Bonas, 1995, Ibid.; U. Bonas, unpublished. Hwang *et al.* (1992, *J Bacteriol* 174: 1923–1931) published the sequence of two genes from *Xanthomonas campestris* pv. *glycines*, designated 'ORF1' and 'ORF2,' that are homologous to hrpD1 and hrpD2 of *X. campestris* pv. *vesicatoria*, respectively.
e. For reviews, see Blair (1995, Ibid.) and Blechoff and Ordal (1992, Ibid.).

may grow. For any new *hrp* genes that may be discovered, we recommend the strict, sequence-alignment-based criterion for use of the '*hrc*' designation until sufficient structural and functional studies can be completed.

Some *hrp* genes are conserved only within subgroups of plant pathogens. One example is the regulatory gene *hrpB* of *R. solanacearum* (Genin *et al.*, 1992, ibid.). This gene, a member of the *araC* family, is present also in pathovars of *X. campestris* (Kamdar *et al.*, 1993, *J Bacteriol* 175: 2017–2025; Kamoun and Kado, 1990, *J Bacteriol* 172: 5165–5172; U. Bonas, unpublished), but absent from the *hrp* gene clusters of *P. syringae* and *E. amylovora*, which contain regulatory genes that are members of the two-component regulatory-system family (Grimm *et al.*, 1995, *Mol Microbiol* 15: 155–165; Grimm and Panopoulos, 1989, *J Bacteriol* 171: 5031–5038; Xiao *et al.*, 1994, ibid.; S. V. Beer, unpublished). As another example, the *hrp* gene clusters of *P. syringae* and *E. amylovora* each contain a homologue of the *Yersinia* gene *yopN* (Bogdanove *et al.*, 1996, ibid.), yet no homologue of this gene has been found in *R. solanacearum* or *X. campestris*. It is noteworthy that the genetic organizations of the *hrp* gene clusters of *X. campestris* and *R. solanacearum* are quite similar to, yet distinct from, those of *P. syringae* and *E. amylovora*, which resemble one another. We will not attempt a nomenclatural revision here for any of the non-*hrc* genes, but we encourage authors, wherever possible, to standardize names for such genes, at least within these subgroups, by using conventional rules for bacterial genetic nomenclature, including priority of publication, as a basis for naming homologues (Demerec *et al.*, 1966, *Genetics* 54: 61–76). Although the same name might be used for different genes across subgroups, standardized names and the similar genetic organizations within the subgroups should greatly facilitate comparative studies and application of information learned in one species to the study of another.

As for the definition of the '*hrp*' designation, it now may include not only genes with a Hrp phenotype, but any gene associated with the Hrp pathway by function, homology, or location within a gene cluster or operon that is essential for the Hrp phenotype. We view use of the '*hrp*' designation in this larger sense as elective rather than mandatory. For example, the designation '*hps*' has been used for Hrp-associated genes shown not to have a strict Hrp phenotype in *R. solanacearum* (Gough *et al.*, 1993, ibid.). In order to minimize confusion in the literature, we propose that this designation be maintained for such genes in this organism and in *X. campestris*. However, for *P. syringae* and the erwiniae, in which gene phenotypes may differ from species to species, we propose a unified nomenclature based on the more inclusive definition of *hrp* genes presented here. We hope that this broadened definition will help us to gain a focussed understanding of the key elements underlying the varied and intricate interactions of bacteria with plants.

For convenience, and because '*hrc*' represents a subset of *hrp* genes, *hrc* and *hrp* genes collectively will be referred to in general discussion as '*hrp*', as in the phrase 'the *hrp* genes of phytopathogenic bacteria.' The combined designation '*hrp/c*' may be used to specify a small group of genes, e.g. 'The genes are arranged co-linearly with their *hrp/c* homologues in *Xanthomonas campestris* pv. *vesicatoria*.' Operons containing *hrc* genes still may be referred to as '*hrp*' operons. When discussing homologues with the same name (*hrp* or *hrc*) from more than one plant pathogen, distinctions can be made where necessary using abbreviations for the names of the different bacteria subscripted to the gene name.

The unified nomenclature for conserved *hrp* genes will benefit research in several ways. It makes the known homologies among plant pathogens explicit. It provides for easy cross-reference to other systems, particulary that of *Yersinia* spp. It facilitates writing and speaking cogently about *hrp* genes. Finally, it transforms a previously confusing jumble of gene names into a well-ordered catalogue, which is an accessible reference not only for *hrp* researchers, but also for those studying other type III secretion systems.

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